

-continued

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ggcaccgagu cggugcuuuu      80

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1. A chimeric single guide RNA molecule (sgRNA), comprising:

- (i) a guide sequence capable of hybridizing to a target sequence in a genomic locus of interest in a eukaryotic cell, wherein the guide sequence has a length of 21 to 23 nucleotides and comprises a 5'-terminal guanosine (G) residue,
- (ii) a tracr-mate sequence capable of hybridizing with a tracr sequence, and
- (iii) a tracr sequence;

wherein the sgRNA is capable of forming a CRISPR-Cas complex with *Staphylococcus aureus* Cas9 (SaCas9).

2. The sgRNA of claim 1, wherein a poly U tract in wild-type tracr-mate sequence, which is located at positions 2-5 starting from 3'-end of the guide sequence, is replaced with poly C.

3. The sgRNA of claim 2, wherein a poly A tract in wild-type tracr sequence, which basepairs with the poly U tract in wild-type tracr-mate sequence, is replaced with poly G.

4. The sgRNA of claim 1, wherein the guide sequence has a length of 21 nucleotides.

5. The sgRNA of claim 1, wherein the guide sequence has a length of 22 nucleotides.

6. The sgRNA of claim 1, wherein the guide sequence has a length of 23 nucleotides.

7. The sgRNA of claim 1, wherein a repeat:anti-repeat duplex formed by hybridization of the tracr-mate sequence and the tracr sequence is truncated compared to that of a wild-type *Staphylococcus aureus* CRISPR-Cas9 system.

8-12. (canceled)

13. The sgRNA of claim 7, wherein the tracr-mate sequence has a length of 14-25 nucleotides.

14-17. (canceled)

18. The sgRNA of claim 1, wherein the tracr sequence has a length of 50-100 nucleotides.

19. The sgRNA of claim 1, wherein the sgRNA comprises at least one loop that comprises an insertion of an RNA sequence capable of binding to an adaptor protein.

20. The sgRNA of claim 19, wherein the inserted RNA sequence is an aptamer.

21. A composition comprising a CRISPR-Cas complex, wherein the CRISPR-Cas complex comprises the sgRNA of claim 1 and a SaCas9 protein.

22. The composition of claim 21, wherein the SaCas9 protein is linked to a heterologous functional domain, optionally wherein the heterologous functional domain is a transcriptional activation domain or a transcriptional repressor domain.

23. (canceled)

24. A composition comprising the sgRNA of claim 20 bound to an adaptor protein.

25. The composition of claim 24, wherein the adaptor protein is linked to a heterologous functional domain, optionally wherein the heterologous functional domain is a transcriptional activation domain or a transcriptional repressor domain.

26. (canceled)

27. A polynucleotide encoding the sgRNA of claim 1.

28. A vector comprising the polynucleotide of claim 27 operably linked to a promoter.

29. The vector of claim 28, further comprising a polynucleotide encoding a SaCas9 protein.

30. A eukaryotic cell comprising the sgRNA of claim 1.

31. A eukaryotic cell transformed with the vector of claim 28.

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